

CLAIMS

1. A process for synthesizing a nucleic acid complementary to a target nucleic acid sequence in a template nucleic acid, which comprises the steps of:

(a) providing a primer comprising in its 3'-end portion a sequence (Ac') which hybridizes a sequence (A) in the 3'-end portion of the target nucleic acid sequence, and in the 5'-side of said sequence (Ac') a sequence (B') which hybridizes the complementary sequence (Bc) of a sequence (B) positioned in the 5'-side of said sequence (A) on the target nucleic acid sequence, wherein

in the absence of an intervening sequence between said sequences (Ac') and (B'), $(X - Y)/X$ is in the range of -1.00 to 1.00, in which X denotes the number of bases in said sequence (Ac'), and Y denotes the number of bases in the region flanked by said sequences (A) and (B) on the target nucleic acid sequence, and

in the presence of an intervening sequence between said sequences (Ac') and (B'), $\{X - (Y - Y')\}/X$ is in the range of -1.00 to 1.00, in which X and Y have the same meanings as above, and Y' denotes the number of bases in said intervening sequence;

(b) providing a template nucleic acid;

(c) annealing said primer to said template nucleic acid and synthesizing a complementary nucleic acid comprising the complementary sequence of said target nucleic acid sequence by primer extension reaction;

(d) hybridizing the sequence (B') positioned in the 5'-side of the complementary nucleic acid synthesized in the step (c) with the sequence (Bc) on the same complementary nucleic acid, thereby allowing the portion of said sequence (A) on the template nucleic acid to be single-stranded; and

(e) annealing another primer having the same sequence as said primer to the single-stranded sequence (A) portion of the template nucleic acid from the step (d) and conducting strand displacement reaction, thereby displacing the complementary nucleic acid synthesized in the step (c) by the complementary nucleic acid newly synthesized with said another primer.

2. The process according to claim 1, wherein the double-stranded nucleic acid obtained by the step (e) is used repeatedly in the step (d).
3. The process according to claim 1, wherein the steps (c), (d) and (e) are carried out in an isothermal condition.
4. The process according to claim 1, wherein a DNA polymerase having strand displacement ability is used.
5. The process according to claim 1, further comprising a step of synthesizing cDNA with a reverse transcriptase when the template nucleic acid is RNA.
6. The process according to claim 1, wherein the steps (c), (d) and (e) are carried out in the presence of a melting temperature adjusting agent.
7. The process according to claim 6, wherein the melting temperature adjusting agent is dimethyl sulfoxide, betains, formamide or glycerol, or a mixture thereof.
8. The process according to claim 1, wherein the target nucleic acid sequence in the template nucleic acid comprises non-natural nucleotide(s).
9. A process for amplifying a target nucleic acid sequence in a double-stranded template nucleic acid, which comprises the steps of:
 - (a) providing a first primer comprising in its 3'-end portion a sequence (Ac') which hybridizes a sequence (A) in the 3'-end portion of the target nucleic acid sequence in the first strand of the double-stranded template nucleic acid, and in the 5'-side of said sequence. (Ac') a sequence (B') which hybridizes the complementary sequence (Bc) of a sequence (B) positioned in the

5'-side of said sequence (A) on said target nucleic acid sequence, wherein

in the absence of an intervening sequence between said sequences (Ac') and (B'), $(X - Y)/X$ is in the range of -1.00 to 1.00, in which X denotes the number of bases in said sequence (Ac'), and Y denotes the number of bases in the region flanked by said sequences (A) and (B) on the target nucleic acid sequence, and

in the presence of an intervening sequence between said sequences (Ac') and (B'), $\{X - (Y - Y')\}/X$ is in the range of -1.00 to 1.00, in which X and Y have the same meanings as above, and Y' denotes the number of bases in said intervening sequence;

(b) providing a second primer comprising in its 3'-end portion a sequence (Cc') which hybridizes a sequence (C) in the 3'-end portion of the target nucleic acid sequence in the second strand of the double-stranded template nucleic acid, and in the 5'-side of said sequence (Cc') a sequence (D') which hybridizes the complementary sequence (Dc) of a sequence (D) positioned in the 5'-side of said sequence (C) on said target nucleic acid sequence, wherein

in the absence of an intervening sequence between said sequences (Cc') and (D'), $(X - Y)/X$ is in the range of -1.00 to 1.00, in which X denotes the number of bases in said sequence (Cc'), and Y denotes the number of bases in the region flanked by said sequences (C) and (D) on the target nucleic acid sequence, and

in the presence of an intervening sequence between said sequences (Cc') and (D'), $\{X - (Y - Y')\}/X$ is in the range of -1.00 to 1.00, in which X and Y have the same meanings as above, and Y' denotes the number of bases in said intervening sequence;

(c) providing a double-stranded template nucleic acid consisting of the first and second template nucleic acids;

(d) annealing said first and second primers to said first and second template nucleic acids, respectively, and synthesizing the first and second complementary nucleic acids comprising the complementary sequence of said target nucleic acid by the primer extension reaction, respectively;

(e) hybridizing the sequences (B') and (D') positioned in the 5'-side

of the first and second complementary nucleic acids synthesized in the step (d) with the sequences (Bc) and (Dc) on the same complementary nucleic acid, respectively, and thereby changing the portions of said sequences (A) and (C) on the first and second template nucleic acids into a single strand, respectively, and
(f) a step of annealing another primers having the same sequence as said primers to the single-stranded sequence (A) and (C) portions of the first and second template nucleic acids from the step (e) and conducting strand displacement reaction, thereby displacing the first and second complementary nucleic acids synthesized in the step (d) by the complementary nucleic acids newly synthesized with said another primers.

10. The process according to claim 9, wherein the double-stranded nucleic acids obtained by the step (f) are used repeatedly in the step (e).

11. The process according to claim 9, wherein the first and second complementary nucleic acids obtained as single strands by the step (f) are used repeatedly as the second and first template nucleic acids, respectively, in the step (d).

12. The process according to claim 9, wherein the steps (d), (e) and (f) are carried out in an isothermal condition.

13. The process according to claim 9, wherein a DNA polymerase having strand displacement ability is used.

14. The process according to claim 9, further comprising a step of synthesizing cDNA with a reverse transcriptase when the template nucleic acid is RNA.

15. The process according to claim 9, wherein the steps (d), (e) and (f) are carried out in the presence of a melting temperature adjusting agent.

16. The process according to claim 15, wherein the melting temperature adjusting agent is dimethyl sulfoxide, betains, formamide or glycerol, or a mixture thereof.

17. The process according to claim 9, wherein the target nucleic acid sequence in the template nucleic acid comprises non-natural nucleotide(s).

18. A primer for synthesizing a nucleic acid complementary to a target nucleic acid sequence in a template nucleic acid, comprising in its 3'-end portion a sequence (Ac') which hybridizes a sequence (A) in the 3'-end portion of the target nucleic acid sequence, and in the 5'-side of said sequence (Ac') a sequence (B') which hybridizes the complementary sequence (Bc) of a sequence (B) positioned in the 5'-side of said sequence (A) on the target nucleic acid sequence, wherein

in the absence of an intervening sequence between said sequences (Ac') and (B'), $(X - Y)/X$ is in the range of -1.00 to 1.00, in which X denotes the number of bases in said sequence (Ac'), and Y denotes the number of bases in the region flanked by said sequences (A) and (B) on the target nucleic acid sequence, and

in the presence of an intervening sequence between said sequences (Ac') and (B'), $\{X - (Y - Y')\}/X$ is in the range of -1.00 to 1.00, in which X and Y have the same meanings as above, and Y' denotes the number of bases in said intervening sequence.

19. A kit for synthesizing a nucleic acid complementary to a target nucleic acid sequence in a template nucleic acid, comprising the primer according to claim 18.

20. A primer set for amplifying a target nucleic acid sequence in a double-stranded template nucleic acid, which comprises:

(a) a first primer comprising in its 3'-end portion a sequence (Ac') which hybridizes a sequence (A) in the 3'-end portion of the target nucleic acid sequence in the first strand of the double-stranded template nucleic acid, and in the 5'-side of said sequence (Ac') a

sequence (B') which hybridizes the complementary sequence (Bc) of a sequence (B) positioned in the 5'-side of said sequence (A) on said target nucleic acid sequence, wherein

in the absence of an intervening sequence between said sequences (Ac') and (B'), $(X - Y)/X$ is in the range of -1.00 to 1.00, in which X denotes the number of bases in said sequence (Ac'), and Y denotes the number of bases in the region flanked by said sequences (A) and (B) on the target nucleic acid sequence, and

in the presence of an intervening sequence between said sequences (Ac') and (B'), $\{X - (Y - Y')\}/X$ is in the range of -1.00 to 1.00, in which X and Y have the same meanings as above, and Y' denotes the number of bases in said intervening sequence; and

(b) a second primer comprising in its 3'-end portion a sequence (Cc') which hybridizes a sequence (C) in the 3'-end portion of the target nucleic acid sequence in the second strand of the double-stranded template nucleic acid, and in the 5'-side of said sequence (Cc') a sequence (D') which hybridizes the complementary sequence (Dc) of a sequence (D) positioned in the 5'-side of said sequence (C) on said target nucleic acid sequence, wherein

in the absence of an intervening sequence between said sequences (Cc') and (D'), $(X - Y)/X$ is in the range of -1.00 to 1.00, in which X denotes the number of bases in said sequence (Cc'), and Y denotes the number of bases in the region flanked by said sequences (C) and (D) on the target nucleic acid sequence, and

in the presence of an intervening sequence between said sequences (Cc') and (D'), $\{X - (Y - Y')\}/X$ is in the range of -1.00 to 1.00, in which X and Y have the same meanings as above, and Y' denotes the number of bases in said intervening sequence.

21. A kit for amplifying a target nucleic acid sequence in a double-stranded template nucleic acid, comprising the primer set according to claim 20.